

Claims

1. A composition, comprising:

5 a plurality of double-stranded RNA (dsRNA) fragments having overlapping sequences, each fragment having a size in the range of 18-30 nt, wherein the composition is formed by enzymatic digestion of one or more large dsRNAs and wherein less than 2nM of the composition is capable of specifically silencing expression of a target
10 gene by at least 65% in transfected COS cells.

2. A composition according to claim 1, wherein the composition is capable of specifically silencing target gene expression by 70%.

15 3. A composition according to claim 1, wherein the composition is capable of specifically silencing target gene expression by 80%.

4. A composition according to claim 1, wherein the dsRNA has a size of at least 100 nt in length.

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5. A composition according to claim 1, wherein the plurality of fragments is at least 5 fragments.

25 6. A composition according to claim 1, wherein the plurality of fragments is at least 10 fragments.

7. A composition according to claim 1, wherein the large dsRNA has a sequence identity with a first portion of a messenger RNA (mRNA) sequence such that the plurality of dsRNA fragments derived
30 therefrom has a greater gene silencing activity at less than 2nM than a second plurality of fragments having sequence identity with a second portion of the mRNA.

8. A composition according to claim 1, wherein the plurality of dsRNA fragments have a greater gene silencing activity at a concentration of less than 2nM than any single fragment in the composition.
9. A composition according to claim 1, wherein the enzymatic digestion is achieved using RNaseIII in a manganese buffer or a mutant RNaseIII.
10. A composition according to claim 1, wherein the target gene encodes Erk1 or Erk2 and the large dsRNA has sequence identity with a portion of mRNA transcribed from the gene.
11. A composition according to claim 1, wherein the target gene encodes Ffluc or Renilla luciferase and the large dsRNA has sequence identity with a portion of mRNA transcribed from the gene.
12. A composition according to claim 1, wherein the fragments are derived from digestion of a plurality of dsRNAs and wherein the plurality of dsRNA have sequence identity with non-contiguous regions of the mRNA.
13. A composition according to claim 1, wherein the fragments are derived from digestion of a plurality of dsRNA wherein the plurality of dsRNA has sequence identity with contiguous regions of the mRNA.

14. A composition according to claim 1, wherein 1nM of the composition is capable of silencing gene expression by at least 70%.

5 15. A method of preparing a composition described in claim 1, comprising:

(a) transcribing at least one RNA molecule having a sequence identity with a portion of a target gene, to form a large dsRNA;

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(b) cleaving the large dsRNA into a mixture of overlapping fragments having a size in the range of 18-30 nt by means of RNaseIII or mutants thereof;

15 (c) determining whether less than 2nM of the large dsRNA can silence at 65% of gene expression of the target gene in COS cells after transfection; and

(d) obtaining the composition described in claim 1.

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16. A method of silencing gene expression; comprising:

(a) cleaving with an enzyme, a large dsRNA having sequence identity with a target gene, wherein the enzyme is RNaseIII or a mutant thereof and the cleavage product is a set of overlapping fragments of dsRNA in which greater than 80% of the fragments have a size of less than about 40nt;

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(b) transfecting cells with the cleavage product of step (a) without size fractionating the product; and

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(c) obtaining at least 65% silencing of expression of the target gene.

17. A method according to claim 16, wherein step (b) further
5 comprises: transfecting cells with less than 2nM of the cleavage
product of step (a).

18. A method according to claim 16, wherein RNase III cleavage is
achieved in the presence of manganese buffer.